SOMATIC HYBRIDS BETWEEN HEAT TOLERANT CABBAGE AND CHINESE CABBAGE

H. Yamagishi(1), M.M. Hossain(2), K. Yonezawa(1)

(1) Kyoto Sangyo University, Kamigamo, Kita-ku, Kyoto 603, Japan
(2) Bangladesh Agricultural Research Institute, Joyde pur, Bangladesh

INTRODUCTION

Chinese cabbage 'Kenshin' (Brassica rapa L. (syn. B. campestris) cv. group Chinese cabbage) and cabbage 'Yoshin' (B.oleracea L. var. capitata) are both indigenous to sub-tropical area of China. They have been used as breeding materials for heat tolerance in Japan and other countries. Interspecific hybrids between these two plants have potentialities of producing new crop cultivars adapted to tropical or sub-tropical conditions(Hossain et al.(1990)). Along with sexually produced hybrids with the aid of embryo rescue method, somatic hybrids produced with cell fusion method will provide new genetic variations useful for the breeding project. On this view, somatic hybrids of 'Kenshin' and 'Yoshin' were produced, and flowering characteristics and seed fertility of the hybrids and leaf morphologies of their offsprings were investigated.

MATERIALS AND METHODS

Seeds of Chinese cabbage 'Kenshin' and cabbage 'Yoshin' were sterilized and sown on medium of Murashige and Skoog (1962) (MS medium). From the aseptically grown plants mesophyll protoplasts were isolated. The protoplast solutions of the two species (1 x 10^6 /ml density) were mixed and treated with 30% polyethylenglycol (PEG 4000). Details of the cell fusion treatment followed the method of Yamagishi et al(1988).

After the cell fusion treatment, protoplasts were cultured with two types of modified MS media, referred to as media A and B, which were supplied with different 2,4-D concentrations of 1 mg/l and 2.5 mg/l respectively, with common application of 1/2 MS (NH_4NO_3=200mg/l), 1% sucrose, 0.5 M mannitol and 0.25 mg/l kinetin. In the beginning 6 weeks, protoplasts were cultured in liquid media. Micro calluses developed were transferred onto callus culture media. After 4 weeks, calluses which had proliferated to diameter larger than 1 mm were transplanted onto regeneration media. All of the protoplast, callus and regeneration cultures were carried out at 20°C with day length of 16-hours after 10 days of all darkness in the beginning of the protoplast culture.

Regenerated plants were transplanted into pots and cultivated in glass house. Temperature in the glass house was kept above 15°C. By external morphology, developed plants were classified into three types, i.e. hybrid, cabbage and Chinese cabbage type. Date of the first flowering was recorded for each plant. When the hybrid plants were at their best flowering, three to five flowers were sampled from each plant to measure petal size (petal length and width) and pollen fertility. The hybrid plants were self-pollinated, cross-pollinated

with Chinese cabbage('Kenryu', an F_1 variety which is closely related to, and has many common characteristics with 'Kenshin') and with cabbage('Yoshin') by bud pollination. At maturity, the number of fertile seeds were counted.

Eleven offspring lines derived with the selfing (SF lines) and 4 lines with the crossing with Chinese cabbage (CF lines) were cultivated in glass house. Leaf length, leaf blade length and leaf width of each plants were measured to obtain leaf size index (leaf length x leaf width) and leaf shape index (leaf width/leaf blade length).

RESULTS

Among 61 plants regenerated, 31 were hybrid type, 29 were cabbage type, and 1 was Chinese cabbage type. The hybrid type plants showed combined leaf morphologies of the parents, and also had PGM isozyme bands of both parents as observed in the hybrids sexually produced by Hossain et al(1989). Twenty one plants among the 31 hybrid type were obtained with medium A. On the other hand, 20 plants among the 29 cabbage type were developed with medium B. The lower 2,4-D concentration of 1 mg/l in the initial protoplast culture media therefore gave a higher production of hybrids, though the total numbers of regenerated plants were similar in the two media (Table 1).

The one Chinese cabbage type plant and all of the hybrid type plants flowered in the glass house. The Chinese cabbage type and 22 out of 31 hybrid type plants flowered within 60 days. Nine hybrid type plants which flowered later than 60 days showed largely different flowering dates ranging from 61 to more than 100 days. In cabbage type, 19 out of 29 plants flowered, though mostly later than 100 days. The remaining 10 plants did not bolt at all. Thus most of the hybrid type plants seemed to be able to flower in temperature higher than 15°C, whereas cabbage type plants appeared to require a long duration of low temperature in order to be vernalized. This flowering characteristic of hybrids is similar to that of Chinese cabbage parent 'Kenshin', coinciding with the result of experiment using sexual hybrids (Hossain et al.(1990)).

Although most of the hybrid type plants had normal pollen fertilities higher than 80%, 5 plants showed fertilities lower than 75%. These five plants flowered later than 60 days (Fig. 1). Petals of hybrid type plants tended to be larger than those of cabbage and Chinese cabbage. Petal length of hybrids was mostly larger than petal width, giving oblong shape of the petal. Three hybrid plants had exceptionally short petal lengths, while having larger petal widths than cabbage. Petal shape of these three was round therefore (Fig. 2).

About 85% of the hybrid type plants produced fertile seeds by self-pollination. The number of seeds per flower varied among the plants, ranging from less than 1.0 to more than 3.0. The hybrids were fertile also in the cross with Chinese cabbage, but infertile in the cross with cabbage (Fig. 3). This one-side crossability has been recognized also in B. napus which were spontaneously developed from B. rapa and B. oleracea. Both the SF and CF lines had larger average leaf size (leaf length x width) than the parental species, suggesting that the hybrid vigor of leaf growth was transmitted to the offsprings. As to leaf shape, SF lines were intermediate of the parental species, and CF lines were closer to Chinese cabbage. Variation coefficients of leaf shape were larger in

SF and CF lines than in the parents, showing larger genetic variations being contained in the offspring lines (Fig. 4).

DISCUSSION

For efficient use of cell fusion technique, it is important to raise the frequency of hybrids in regenerated plants. A number of methods e.g., cell sorting method (Glimelius et al.(1986)), pretreatment of IOA (Terada et al.(1987)) and so on, have been exploited until now, but were not used in this experiment. Instead, two different concentrations of growth regulator 2,4-D were tested for the initial protoplast culture. A higher frequency of hybrid plants was obtained in the medium with lower 2,4-D concentration, suggesting that the frequency of hybrids among regenerated plants could be increased by optimizing growth regulator concentrations.

Somatic hybrids could flower under relatively high temperature and most of them produced fertile seeds with self pollination. The somatic hybrids therefore is expected to bear enough seeds even in tropical or subtropical areas. They also may be used as a bridge species for introducing useful genes of cabbage into Chinese cabbage. Offspring lines of the somatic hybrids showed better growth of leaves and larger variation of leaf shape than cabbage and Chinese cabbage. Thus, new promising cultivars and/or genetic stocks are expected to be selected in the offsprings.

SUMMARY

Somatic hybrids between heat tolerant cabbage 'Yoshin' and Chinese cabbage 'Kenshin' were produced by means of cell fusion. A higher frequency of the somatic hybrids was obtained with a lower concentration (1 mg/l) of 2,4-D in the initial protoplast culture medium. Most of the hybrid plants flowered early and had normal pollen fertility and larger petals than parental species. They produced fertile seeds by selfing and crossing with Chinese cabbage, but infertile by crossing with cabbage. Offsprings of the hybrids had larger size of leaves and larger heterogeneity of leaf shape than the parents.

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Table 1 Protoplast culture media and types of regenerated plants.

	Type of plants			Total
Medium	Hybrid	Cabbage	Chinese cabbage	number
A	20	9	0	29
B	11	20	1	32

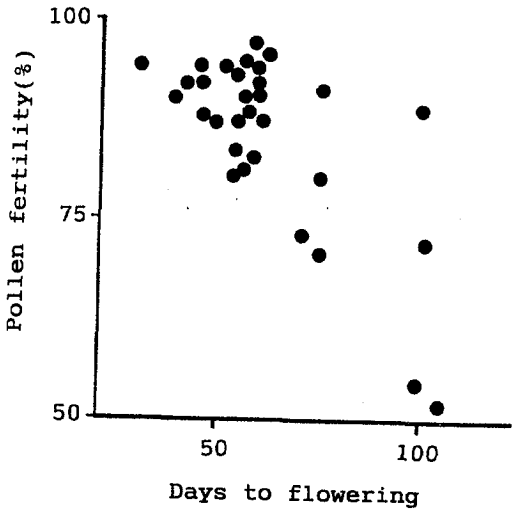

Fig. 1 Relationship between flowering date and pollen fertility of somatic hybrids.

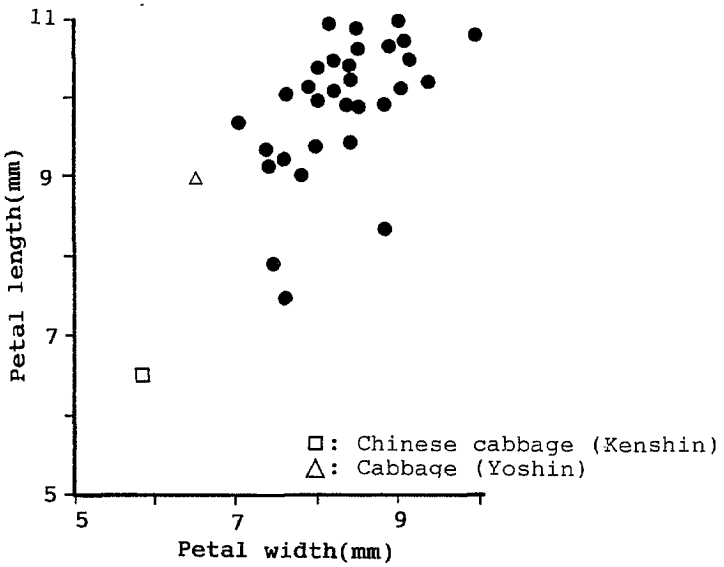

Fig. 2 Petal width and length of somatic hybrids between 'Kenshin' and 'Yoshin'.

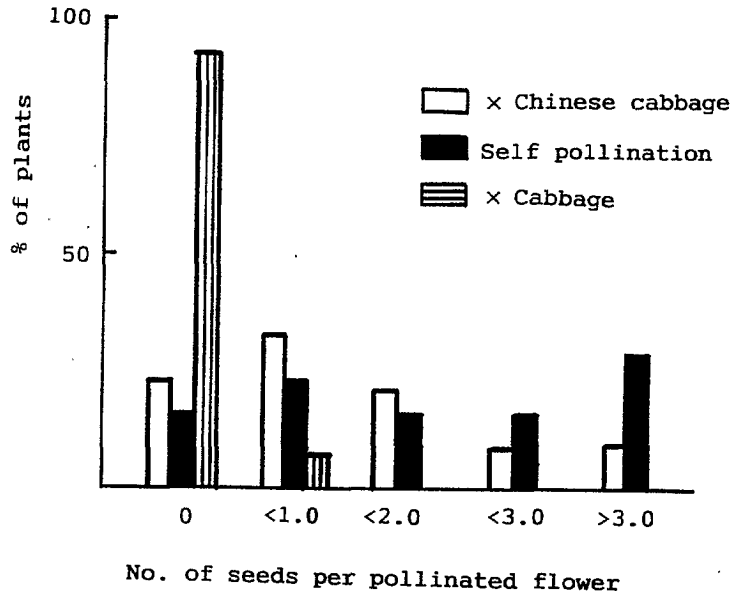

Fig. 3 Seed fertility of somatic hybrids by self-pollination, crosses with Chinese cabbage and cabbage.

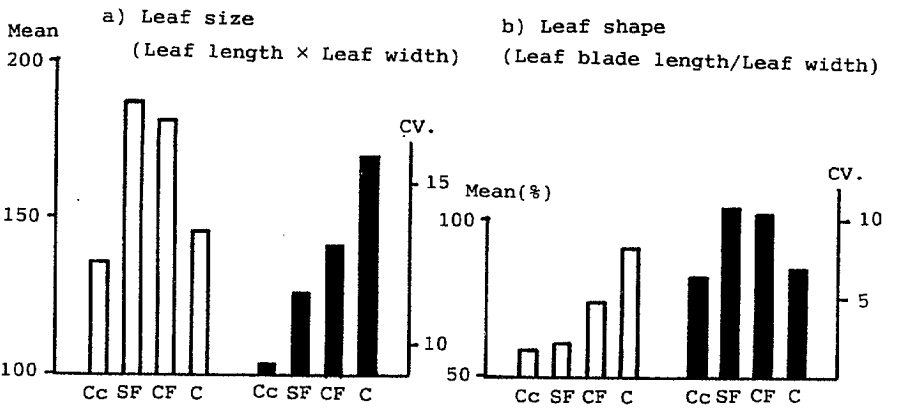

Fig. 4 Leaf morphologies and their variations within lines in offsprings of somatic hybrids.
Cc; Chinese cabbage 'Kenshin'. C; Cabbage 'Yoshin'.
SF; Offspring lines obtained by selfing.
CF; Offspring lines obtained by crossing with Chinese cabbage.